32 Airway hyperresponsiveness in FVB/N ΔF508 cystic fibrosis transmembrane conductance regulator mice

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Introduction: Airway hyperresponsiveness (AHR) is a component of CF lung disease found in up to 40% of patients. We have previously shown that CFTR (BALB $Cftr^{tm1UNC}$) knockout mice present AHR to methacholine challenge. In the present study, we investigated whether the more clinically relevant FVB/N ΔF508 CFTR mouse model develops AHR and then evaluated inflammation and histological measures associated with the response.
Approach: At 12 weeks old, $Cftr^{tm1Eur}$ mice (ΔF508) were placed on the FlexiVent small mammal ventilator and baseline measurements were recorded. Increasing doses of methacholine were administered allowing AHR to be measured. Following AHR measurements the mice were sacrificed and tissues collected for histological and bronchoalveolar lavage analysis.
Results: $Cftr^{tm1Eur}$ mice presented with an increase in both resistance and elastance baseline measurements while also having enhanced AHR to methacholine challenge when compared to wildtype littermates. The bronchoalveolar lavage of $Cftr^{tm1Eur}$ mice included significantly increased levels of Interleukin 12p40 (IL12p40) compared to controls, while other cytokines including IL4, IL5 and IFN-g showed no difference. Furthermore, no differences in bronchoalveolar cell number or differential were detected between CF and wildtype controls. Finally, many indicators of asthma airway hyperresponsiveness including eosinophils, tissue neutrophil, mucous, goblet cells and smooth muscle actin were similar between mice grouped by Cftr genotype.
Conclusion: We conclude that the pulmonary phenotype of $Cftr^{tm1Eur}$ mice includes AHR and enhanced IL12p40 in the absence of many of the key indicators seen in asthma.

33 Hypoxia reduces internalization of *Pseudomonas aeruginosa* in pulmonary epithelial cells

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Pseudomonas aeruginosa is the predominant bacterial pathogen in cystic fibrosis (CF) and grows within a hypoxic micro-environment in the CF lung causing chronic infection and associated inflammation. In this study we have investigated the impact of hypoxia on the infection of pulmonary epithelial cells with *P. aeruginosa*. In an in vitro model for bacterial infection, we studied the influence of hypoxia on the uptake of *P. aeruginosa*into pulmonary epithelial cells. Hypoxia significantly reduced the internalisation of *P. aeruginosa* (ATCC 27853) into the cells by 95.7% ($p = 0.02$), resulting in diminished levels of epithelial cell death when compared to normoxic conditions. Furthermore, internalization of clinical CF strains of *P. aeruginosa* was decreased in pulmonary epithelial cells treated with the hyposxia mimetic DMOG. Although the regulatior of innate immune responses, NF-kappaB was increased in *P. aeruginosa* infected hypoxic cells, silencing of NF-kappaB did not increase the number of internalized bacteria under low oxygen tensions. Silencing of HIF-1alpha however, partially normalized decreased internalization levels of *P. aeruginosa* in hypoxia ($p = 0.046$). Increased active RhoA was also partially responsible for the decrease in internalization of *P. aeruginosa* in pulmonary epithelial cells under hypoxic conditions ($p = 0.01$). These data indicate that under conditions of hypoxia *P. aeruginosa* infection is decreased in a manner dependent on HIF-1alpha and RhoA. This effect could be reproduced using the hypoxia mimetic DMOG. Further investigations of signaling molecules participating in this response will reveal new targets for anti-inflammatory therapy in CF.

34 Airflow and nebulized hypertonic saline decrease CBF in vitro

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Nebulized hypertonic saline (HS) is widely used for the treatment of CF, but data about its influence on ciliary beat frequency (CBF) are scarce. High flow nasal cannula is used as respiratory support in preterm infants, but again its influence on CBF is unknown.
Aim: To study the effect of airflow and nebulized HS on CBF in vitro.
Methods: Nasal epithelial cells from non-CF surgery specimens were treated with 0.1% Pronase solution and cultured as a monolayer. On day 4 to 6, after removal of the cell culture medium (DMEM-Ham's F12, Life Technologies, with AB and Ultroser G), the confluent cell monolayers were for 10 minutes exposed to air flow or to nebulization of 6% HS in an aerosol chamber, using a Pari boy and a Pari LC star nebulizer set. The CBF was measured before exposure and at various points during and after exposure, up to 60 min. Images were acquired by a MotionScope high-speed camera mounted on an inverted Olympus microscope. CBF was calculated with Matlab software. Removal of medium alone and nebulization with medium were used as control.
Results: Exposure to dry air ($n = 13$) resulted in a significant ($p < 0.0001$) CBF decrease, with minimum one minute post exposure, from 6.10 Hz (sem 0.81) to 5.34 Hz (sem 0.70). Nebulization of HS ($n = 6$) resulted in a significant (p 0.02) CBF decrease, with minimum 30 minutes post nebulization, from 6.23 Hz (sem 0.92) to 5.28 Hz (sem 0.84). Removal of or nebulization with culture medium alone did not significantly influence CBF.
Conclusion: *In vitro* dry air exposure and HS nebulization result in a CBF decrease. The relevance of this finding to daily use of nasal flow and /or HS nebulization should be further evaluated.

35 Modulation of ion secretion by bile acids in Calu-3 airway epithelial cells

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Gastroesophageal reflux is common in Cystic Fibrosis (CF) patients and increases the risk of aspirating (duodeno)-gastric contents into the lungs [1]. Bile acids have been shown to acutely stimulate or chronically inhibit epithelial Cl^- secretion in the colon [2]. Here we investigated the effect of the unconjugated bile acid, deoxycholic acid (DCA, 25 μM), and the conjugated bile acid tauroDCA (TDCA, 25 μM) on the secretory response to carbachol (100 μM) in cultured monolayers of Calu-3 airway epithelial cells mounted in Ussing chambers. We found that acute basolateral treatment of Calu-3 cells with TDCA produced a 15-fold increase in the basal electrogenic transepithelial ion transport measured as short-circuit current (Isc) ($n = 4$, $p = 0.0028$). Acute treatment of Calu-3 cells with DCA resulted in an attenuation of the Isc response stimulated by carbachol (apical DCA 47±17% $n = 5$, $p = 0.0467$, basolateral DCA 50±13% $n = 2$, p = n.s). Longer treatment for 24hr of Calu-3 cells with DCA apically or TDCA bi-laterally did not produce a significant attenuation of the carbachol-secretory response. Western blotting showed that Calu-3 cells express the bile acid receptor Farsenoid X Receptor (FXR). Thus bile acids exhibit a temporal dependence and sideness in modulating airway cell ion transport, possibly via the nuclear FXR.

Reference(s)

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